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       CHIN, WIA-PAN
       CHEN, LIN
       FITZPATRICK, PAUL A.
       HENSTRAND, JOHN M.
       WENDTH, DAN J.
       ZECHERLE, GARY N.
       STARR, CHRISTOPEHER M.
       KAKKIS, EMIL D.

<120> METHODS FOR PRODUCING AND PURIFYING RECOMBINANT ALPHA-L-
      IDURONIDASE

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<151> 2000-11-09

<150> 09/439,923
<151> 1999-11-12

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ctctccttgt ctgagccata actcttcttt ctaccttcca gtgaacacct tcccacccca    1260
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ggagctgtcc ccagaaagct acctagtgtt tgagccccta aaggagccag gcgagggagc    1440
caccacctac ctggtgacaa gcgtgttgcg tgtatcagct gaaagcttga tatcgaattc    1500
cggaggcgga accggcagtg cagcccgaag ccccgcagtc cccgagcacg cgtggcc atg    1560
                                                                  Met
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cgt ccc ctg cgc ccc cgc gcc gcg ctg ctg gcg ctc ctg gcc tcg ctc    1608
Arg Pro Leu Arg Pro Arg Ala Ala Leu Leu Ala Leu Leu Ala Ser Leu
         5                   10                  15
```

```
ctg gcc gcg ccc ccg gtg gcc ccg gcc gag gcc ccg cac ctg gtg cat    1656
Leu Ala Ala Pro Pro Val Ala Pro Ala Glu Ala Pro His Leu Val His
        20                  25                  30
```

```
gtg gac gcg gcc cgc gcg ctg tgg ccc ctg cgg cgc ttc tgg agg agc    1704
Val Asp Ala Ala Arg Ala Leu Trp Pro Leu Arg Arg Phe Trp Arg Ser
        35                  40                  45
```

```
aca ggc ttc tgc ccc ccg ctg cca cac agc cag gct gac cag tac gtg    1752
Thr Gly Phe Cys Pro Pro Leu Pro His Ser Gln Ala Asp Gln Tyr Val
50                  55                  60                  65
```

```
ctc agc tgg gac cag cag ctc aac ctc gcc tat gtg ggc gcc gtc cct    1800
Leu Ser Trp Asp Gln Gln Leu Asn Leu Ala Tyr Val Gly Ala Val Pro
                70                  75                  80
```

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cac cgc ggc atc aag cag gtc cgg acc cac tgg ctg ctg gag ctt gtc    1848
His Arg Gly Ile Lys Gln Val Arg Thr His Trp Leu Leu Glu Leu Val
                85                  90                  95
```

```
acc acc agg ggg tcc act gga cgg ggc ctg agc tac aac ttc acc cac    1896
Thr Thr Arg Gly Ser Thr Gly Arg Gly Leu Ser Tyr Asn Phe Thr His
        100                 105                 110
```

```
ctg gac ggg tac ctg gac ctt ctc agg gag aac cag ctc ggg ttt gag    1944
Leu Asp Gly Tyr Leu Asp Leu Leu Arg Glu Asn Gln Leu Gly Phe Glu
        115                 120                 125
```

```
ctg atg ggc agc gcc tcg ggc cac ttc act gac ttt gag gac aag cag    1992
Leu Met Gly Ser Ala Ser Gly His Phe Thr Asp Phe Glu Asp Lys Gln
130                 135                 140                 145
```

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cag gtg ttt gag tgg aag gac ttg gtc tcc agc ctg gcc agg aga tac    2040
Gln Val Phe Glu Trp Lys Asp Leu Val Ser Ser Leu Ala Arg Arg Tyr
                150                 155                 160
```

```
atc ggt agg tac gga ctg gcg cat gtt tcc aag tgg aac ttc gag acg    2088
Ile Gly Arg Tyr Gly Leu Ala His Val Ser Lys Trp Asn Phe Glu Thr
                165                 170                 175
```

```
tgg aat gag cca gac cac cac gac ttt gac aac gtc tcc atg acc atg    2136
Trp Asn Glu Pro Asp His His Asp Phe Asp Asn Val Ser Met Thr Met
                180                 185                 190
```

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caa ggc ttc ctg aac tac tac gat gcc tgc tcg gag ggt ctg cgc gcc    2184
Gln Gly Phe Leu Asn Tyr Tyr Asp Ala Cys Ser Glu Gly Leu Arg Ala
        195                 200                 205
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gcc agc ccc gcc ctg cgg ctg gga ggc ccc ggc gac tcc ttc cac acc        2232
Ala Ser Pro Ala Leu Arg Leu Gly Gly Pro Gly Asp Ser Phe His Thr
210               215               220                   225

cca ccg cga tcc ccg ctg agc tgg ggc ctc ctg cgc cac tgc cac gac        2280
Pro Pro Arg Ser Pro Leu Ser Trp Gly Leu Leu Arg His Cys His Asp
                    230               235               240

ggt acc aac ttc ttc act ggg gag gcg ggc gtg cgg ctg gac tac atc        2328
Gly Thr Asn Phe Phe Thr Gly Glu Ala Gly Val Arg Leu Asp Tyr Ile
                245               250                   255

tcc ctc cac agg aag ggt gcg cgc agc tcc atc tcc atc ctg gag cag        2376
Ser Leu His Arg Lys Gly Ala Arg Ser Ser Ile Ser Ile Leu Glu Gln
            260               265               270

gag aag gtc gtc gcg cag cag atc cgg cag ctc ttc ccc aag ttc gcg        2424
Glu Lys Val Val Ala Gln Gln Ile Arg Gln Leu Phe Pro Lys Phe Ala
        275               280               285

gac acc ccc att tac aac gac gag gcg gac ccg ctg gtg ggc tgg tcc        2472
Asp Thr Pro Ile Tyr Asn Asp Glu Ala Asp Pro Leu Val Gly Trp Ser
290               295                   300               305

ctg cca cag ccg tgg agg gcg gac gtg acc tac gcg gcc atg gtg gtg        2520
Leu Pro Gln Pro Trp Arg Ala Asp Val Thr Tyr Ala Ala Met Val Val
                310               315               320

aag gtc atc gcg cag cat cag aac ctg cta ctg gcc aac acc acc tcc        2568
Lys Val Ile Ala Gln His Gln Asn Leu Leu Leu Ala Asn Thr Thr Ser
            325               330               335

gcc ttc ccc tac gcg ctc ctg agc aac gac aat gcc ttc ctg agc tac        2616
Ala Phe Pro Tyr Ala Leu Leu Ser Asn Asp Asn Ala Phe Leu Ser Tyr
        340               345               350

cac ccg cac ccc ttc gcg cag cgc acg ctc acc gcg cgc ttc cag gtc        2664
His Pro His Pro Phe Ala Gln Arg Thr Leu Thr Ala Arg Phe Gln Val
        355               360               365

aac aac acc cgc ccg ccg cac gtg cag ctg ttg cgc aag ccg gtg ctc        2712
Asn Asn Thr Arg Pro Pro His Val Gln Leu Leu Arg Lys Pro Val Leu
370               375               380               385

acg gcc atg ggg ctg ctg gcg ctg ctg gat gag gag cag ctc tgg gcc        2760
Thr Ala Met Gly Leu Leu Ala Leu Leu Asp Glu Glu Gln Leu Trp Ala
                390               395               400

gaa gtg tcg cag gcc ggg acc gtc ctg gac agc aac cac acg gtg ggc        2808
Glu Val Ser Gln Ala Gly Thr Val Leu Asp Ser Asn His Thr Val Gly
            405               410               415

gtc ctg gcc agc gcc cac cgc ccc cag ggc ccg gcc gac gcc tgg cgc        2856
Val Leu Ala Ser Ala His Arg Pro Gln Gly Pro Ala Asp Ala Trp Arg
        420               425               430

gcc gcg gtg ctg atc tac gcg agc gac gac acc cgc gcc cac ccc aac        2904
Ala Ala Val Leu Ile Tyr Ala Ser Asp Asp Thr Arg Ala His Pro Asn
        435               440               445
```

-45-

```
cgc agc gtc gcg gtg acc ctg cgg ctg cgc ggg gtg ccc ccc ggc ccg          2952
Arg Ser Val Ala Val Thr Leu Arg Leu Arg Gly Val Pro Pro Gly Pro
450             455             460             465

ggc ctg gtc tac gtc acg cgc tac ctg gac aac ggg ctc tgc agc ccc          3000
Gly Leu Val Tyr Val Thr Arg Tyr Leu Asp Asn Gly Leu Cys Ser Pro
                470             475             480

gac ggc gag tgg cgg cgc ctg ggc cgg ccc gtc ttc ccc acg gca gag          3048
Asp Gly Glu Trp Arg Arg Leu Gly Arg Pro Val Phe Pro Thr Ala Glu
            485             490             495

cag ttc cgg cgc tag cgc gcg gct gag gac ccg gtg gcc gcg gcg ccc          3096
Gln Phe Arg Arg  *  Arg Ala Ala Glu Asp Pro Val Ala Ala Ala Pro
        500             505             510

cgc ccc tta ccc gcc ggc ggc cgc ctg agg ctg cgc ccc gcg ctg cgg          3144
Arg Pro Leu Pro Ala Gly Gly Arg Leu Arg Leu Arg Pro Ala Leu Arg
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ctg ccg tcg ctt ttg ctg gtg cac gtg tgt gcg cgc ccc gag aag ccg          3192
Leu Pro Ser Leu Leu Leu Val His Val Cys Ala Arg Pro Glu Lys Pro
    530             535             540

ccc ggg cag gtc acg cgg ctc cgc gcc ctg ccc ctg acc caa ggg cag          3240
Pro Gly Gln Val Thr Arg Leu Arg Ala Leu Pro Leu Thr Gln Gly Gln
545             550             555             560

ctg gtt ctg gtc tgg tcg gat gaa cac gtg ggc tcc aag tgc ctg tgg          3288
Leu Val Leu Val Trp Ser Asp Glu His Val Gly Ser Lys Cys Leu Trp
            565             570             575

aca tac gag atc cag ttc tct cag gac ggt aag gcg tac acc ccg gtc          3336
Thr Tyr Glu Ile Gln Phe Ser Gln Asp Gly Lys Ala Tyr Thr Pro Val
            580             585             590

agc agg aag cca tcg acc ttc aac ctc ttt gtg ttc agc cca gac aca          3384
Ser Arg Lys Pro Ser Thr Phe Asn Leu Phe Val Phe Ser Pro Asp Thr
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ggt gct gtc tct ggc tcc tac cga gtt cga gcc ctg gac tac tgg gcc          3432
Gly Ala Val Ser Gly Ser Tyr Arg Val Arg Ala Leu Asp Tyr Trp Ala
610             615             620

cga cca ggc ccc ttc tcg gac cct gtg ccg tac ctg gag gtc cct gtg          3480
Arg Pro Gly Pro Phe Ser Asp Pro Val Pro Tyr Leu Glu Val Pro Val
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cca aga ggg ccc cca tcc ccg ggc aat cca tgagcctgtg ctgagcccca           3530
Pro Arg Gly Pro Pro Ser Pro Gly Asn Pro
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-46-

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His Val Asp Ala Ala Arg Ala Leu Trp Pro Leu Arg Arg Phe Trp Arg
            35                  40                  45
Ser Thr Gly Phe Cys Pro Pro Leu Pro His Ser Gln Ala Asp Gln Tyr
        50                  55                  60
Val Leu Ser Trp Asp Gln Gln Leu Asn Leu Ala Tyr Val Gly Ala Val
65                  70                  75                  80
Pro His Arg Gly Ile Lys Gln Val Arg Thr His Trp Leu Leu Glu Leu
                85                  90                  95
Val Thr Thr Arg Gly Ser Thr Gly Arg Gly Leu Ser Tyr Asn Phe Thr
                100                 105                 110
His Leu Asp Gly Tyr Leu Asp Leu Leu Arg Glu Asn Gln Leu Gly Phe
            115                 120                 125
Glu Leu Met Gly Ser Ala Ser Gly His Phe Thr Asp Phe Glu Asp Lys
        130                 135                 140
```

-47-
```

Gln Gln Val Phe Glu Trp Lys Asp Leu Val Ser Ser Leu Ala Arg Arg
145                 150                 155                 160
Tyr Ile Gly Arg Tyr Gly Leu Ala His Val Ser Lys Trp Asn Phe Glu
                165                 170                 175
Thr Trp Asn Glu Pro Asp His His Asp Phe Asp Asn Val Ser Met Thr
            180                 185                 190
Met Gln Gly Phe Leu Asn Tyr Tyr Asp Ala Cys Ser Glu Gly Leu Arg
        195                 200                 205
Ala Ala Ser Pro Ala Leu Arg Leu Gly Gly Pro Gly Asp Ser Phe His
    210                 215                 220
Thr Pro Pro Arg Ser Pro Leu Ser Trp Gly Leu Leu Arg His Cys His
225                 230                 235                 240
Asp Gly Thr Asn Phe Phe Thr Gly Glu Ala Gly Val Arg Leu Asp Tyr
                245                 250                 255
Ile Ser Leu His Arg Lys Gly Ala Arg Ser Ser Ile Ser Ile Leu Glu
            260                 265                 270
Gln Glu Lys Val Val Ala Gln Gln Ile Arg Gln Leu Phe Pro Lys Phe
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Ala Asp Thr Pro Ile Tyr Asn Asp Glu Ala Asp Pro Leu Val Gly Trp
    290                 295                 300
Ser Leu Pro Gln Pro Trp Arg Ala Asp Val Thr Tyr Ala Ala Met Val
305                 310                 315                 320
Val Lys Val Ile Ala Gln His Gln Asn Leu Leu Leu Ala Asn Thr Thr
                325                 330                 335
Ser Ala Phe Pro Tyr Ala Leu Leu Ser Asn Asp Asn Ala Phe Leu Ser
            340                 345                 350
Tyr His Pro His Pro Phe Ala Gln Arg Thr Leu Thr Ala Arg Phe Gln
        355                 360                 365
Val Asn Asn Thr Arg Pro Pro His Val Gln Leu Leu Arg Lys Pro Val
    370                 375                 380
Leu Thr Ala Met Gly Leu Leu Ala Leu Leu Asp Glu Glu Gln Leu Trp
385                 390                 395                 400
Ala Glu Val Ser Gln Ala Gly Thr Val Leu Asp Ser Asn His Thr Val
                405                 410                 415
Gly Val Leu Ala Ser Ala His Arg Pro Gln Gly Pro Ala Asp Ala Trp
            420                 425                 430
Arg Ala Ala Val Leu Ile Tyr Ala Ser Asp Asp Thr Arg Ala His Pro
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Asn Arg Ser Val Ala Val Thr Leu Arg Leu Arg Gly Val Pro Pro Gly
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Pro Gly Leu Val Tyr Val Thr Arg Tyr Leu Asp Asn Gly Leu Cys Ser
465                 470                 475                 480
Pro Asp Gly Glu Trp Arg Arg Leu Gly Arg Pro Val Phe Pro Thr Ala
                485                 490                 495
Glu Gln Phe Arg Arg Arg Ala Ala Glu Asp Pro Val Ala Ala Ala Pro
            500                 505                 510
Arg Pro Leu Pro Ala Gly Gly Arg Leu Arg Leu Arg Pro Ala Leu Arg
        515                 520                 525
Leu Pro Ser Leu Leu Leu Val His Val Cys Ala Arg Pro Glu Lys Pro
    530                 535                 540
Pro Gly Gln Val Thr Arg Leu Arg Ala Leu Pro Leu Thr Gln Gly Gln
545                 550                 555                 560
Leu Val Leu Val Trp Ser Asp Glu His Val Gly Ser Lys Cys Leu Trp
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Thr Tyr Glu Ile Gln Phe Ser Gln Asp Gly Lys Ala Tyr Thr Pro Val
            580                 585                 590
Ser Arg Lys Pro Ser Thr Phe Asn Leu Phe Val Phe Ser Pro Asp Thr
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Gly Ala Val Ser Gly Ser Tyr Arg Val Arg Ala Leu Asp Tyr Trp Ala
    610                 615                 620
Arg Pro Gly Pro Phe Ser Asp Pro Val Pro Tyr Leu Glu Val Pro Val

-48-

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                          645                  650

1
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